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Stipular Bud Development in *Danaea wendlandii* (Marattiaceae)

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It has long been known that large numbers of buds are produced around the leaf bases of sporophytes in the Marattiales (Gwynne-Vaughan, 1905), and extensive use has been made of this phenomenon in propagation of *Angiopteris* and *Marattia* (West, 1917). *Danaea* is a neotropical genus in the Marattiaceae comprising about 20 species, all of which are limited to the herbaceous layer of the tropical rain forest (Tryon & Tryon, 1982). West (1917) documented the presence of arrested and expanding buds at bases of petioles of *Danaea nodosa*, and asexual propagation by means of leaf-tip budding is common in several species, including *D. wendlandii* Reichenb. (Tryon & Tryon, 1982).

In demographic and developmental studies of *D. wendlandii* (Sharpe, 1988; Sharpe & Jernstedt, 1990a, 1990b) examination of more than five hundred harvested plants showed that expansion of leaf-tip buds routinely followed leaf expansion. However, not even a single example of petiolar bud development was observed in field-collected specimens of this species (Sharpe, unpublished data). Because this was unexpected, an investigation of petiolar budding potential in this species was undertaken.

There are several patterns of bud formation in ferns (Troop & Mickel, 1968). This may, in part, account for the conflicting results in the literature of experimental studies of bud development in ferns (White, 1979). The relatively slow growth rates of ferns result in more gradual budding responses and require much longer experiments than in many seed plants (Croxdale, 1976).

The objectives of this study were (1) to determine if bud development could be forced to occur in *D. wendlandii* using a variety of experimental treatments and (2) to examine the location and frequency of arrested and expanded buds at the bases of petioles of *D. wendlandii*.

MATERIAL AND METHODS

Description of species.—Adult plants of *Danaea wendlandii* were used for our experiments. These plants form a compact rosette of approximately six leaves, each of which attains a mean length of about 21 cm (Sharpe, 1988). Leaves emerge from the protective enclosure of petiole base stipules, which are characteristic of the Marattiaceae (Bower, 1908). Each sterile adult leaf develops

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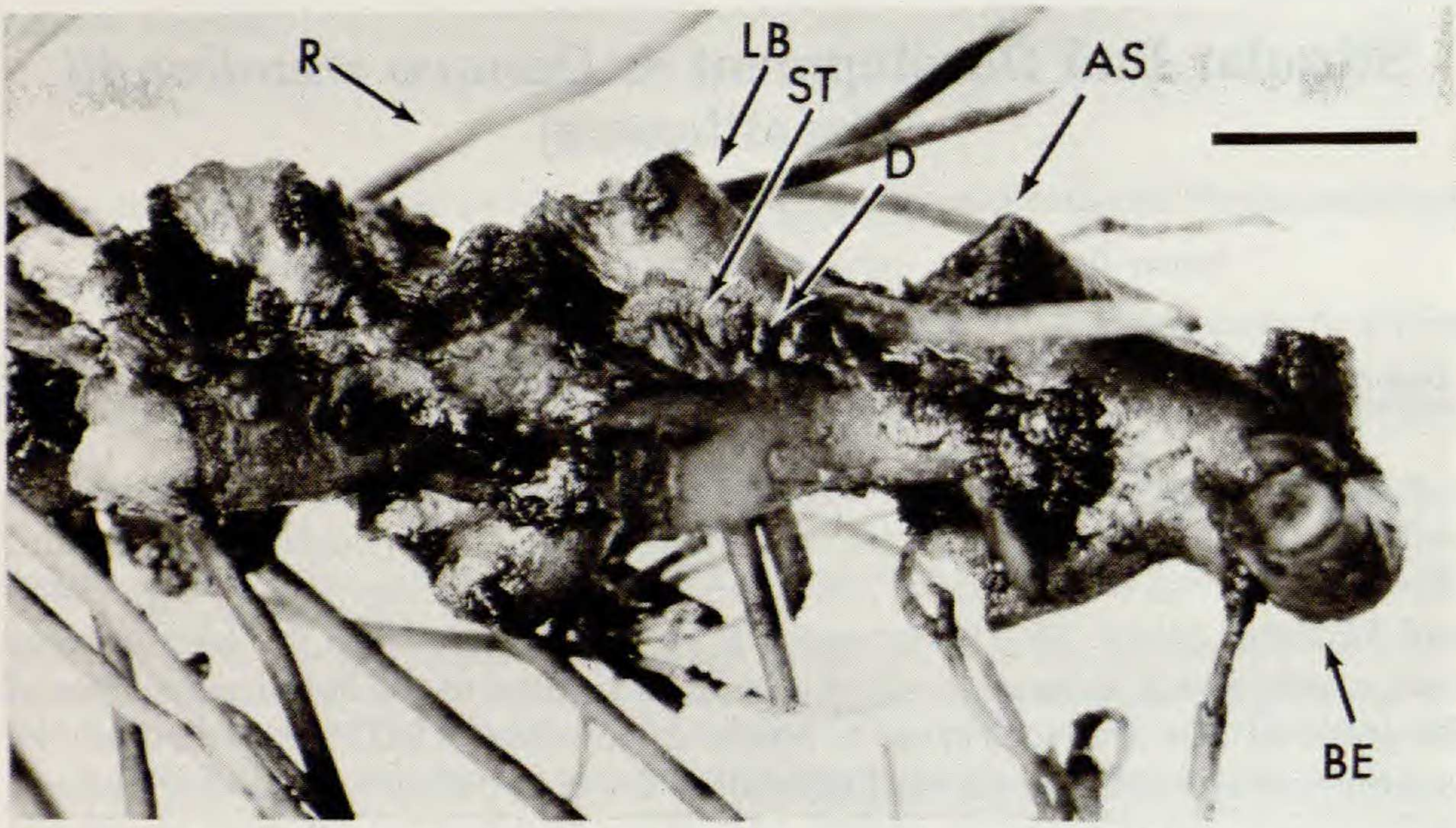


FIG. 1. Distal portion of a rhizome of an adult sterile plant of *Danaea wendlandii*. Scale bar = 1 cm. The basal end of the rhizome (BE) and roots (R) are shown. As each leaf abscised in this portion of the rhizome, an abscission scar (AS) remained. Each leaf base (LB) remains attached to the rhizome with two associated stipules (ST). Each stipule is attached to the petiole base on its abaxial surface (proximal stipule margin, not seen) and its adaxial surface (distal stipule margin, D).

a terminal bud, which produces roots and leaves prior to leaf abscission (Sharpe, 1988). Leaf abscission occurs approximately four months after emergence for fertile leaves and approximately three years after emergence for sterile leaves (Sharpe & Jernstedt, 1990a). The abscission zone on the petiole of *D. wendlandii* is about a centimeter above the point of attachment of the leaf base to the rhizome (Fig. 1). The mean length of a rhizome of an adult plant is about 4 cm (Sharpe, 1988) and has an average of 21 nodes (Table 1). At each node where the leaf has abscised, a persistent leaf base consisting of a short section of the petiole and its two associated stipules remains attached to the rhizome (Figs. 1, 2).

Experimental treatments.—Adult plants of *Danaea wendlandii* were harvested from primary rain forest at the La Selva Biological Station in Costa Rica in September 1986. Within 48 hours, each plant was transplanted to a greenhouse at the University of Georgia. The transplanted sporophytes were grown in a soil mix commonly used for epiphytes (B. McAlpin, personal communication). High humidity and low light levels were maintained in a growth chamber created by enclosing a 3 m × 1 m greenhouse bench with 0.5 m wooden sides. The top of the chamber was sealed with clear plastic and covered with two layers of shade cloth. Plants were watered daily with tap water.

The total counts of nodes and leaves on each experimental plant ($N = 72$) were recorded prior to treatment. Four different treatments were used: apex

TABLE 1. Comparison of the effects of different shoot apex removal treatments on the development of stipular buds on petiole bases of *Danaea wendlandii*. Buds were scored visually without dissection of the rhizome. Counts of nodes with buds are shown as per plant means ± standard error, with ranges in parentheses. There were 18 plants per treatment. Decapitated plants had the shoot apex removed but no further treatment. Lanolin and 1% IAA in lanolin replaced decapitated apices in those treatments. Mean total number of nodes per plant is also shown for each treatment. Means followed by the same letters were not statistically different (Mann-Whitney U-test, $P > 0.05$).

Treatment	Number of nodes with expanded stipular buds	Total node count per plant
Intact	0.1 ± 0.05 a (0−1)	21.7 ± 0.81
Decapitated only	2.7 ± 0.54 b (0−7)	21.4 ± 0.61
Decapitated plus plain lanolin	3.8 ± 0.94 b (0−14)	20.3 ± 1.01
Decapitated plus 1% IAA in lanolin	1.6 ± 0.58 b (0−10)	19.0 ± 0.97

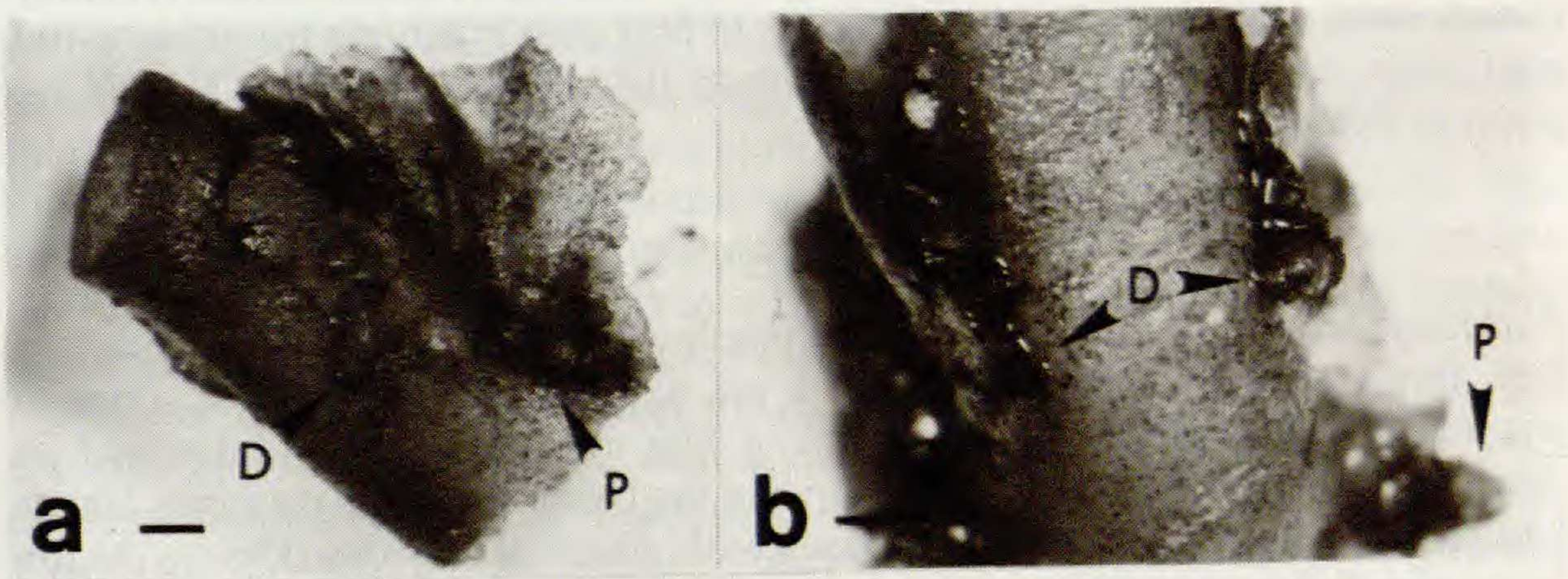


FIG. 2. Macroscopic views of the petiole base of an abscised leaf of *Danaea wendlandii*. Scale bar = 1 mm. A. Side view of petiole base with abscission scar at top and abaxial surface toward the lower left. Proximal (P) and distal (D) stipule margins indicated by arrows. B. View of abaxial side of petiole base with uppermost two buds (D) located at distal stipule margins, and lower right bud (P) located at proximal stipule margin.

removed (decapitated), apex removed and replaced with plain lanolin paste, apex removed and replaced with lanolin containing 1% (w/v) auxin (IAA), and apex left intact (Cutter, 1978). For all treatments involving decapitation, the shoot apex and coiled croziers were removed, but all expanded leaves were left intact.

Treatments were initially applied on a staggered schedule starting in November 1986. Every three weeks for four months 12 plants were randomly selected, and each treatment applied to three plants. Every three weeks during this period, previously treated apices were swabbed and lanolin paste (with or without IAA) was re-applied.

Harvest and bud scoring.—All 72 plants were harvested and scored at the end of eight months, in July 1987. Expanded buds visible on rhizomes or leaf bases were counted. Plants were then returned to the greenhouse. Two months later, eight of the re-planted sporophytes from various treatments were harvested, dissected, and each petiole base examined under a dissecting microscope.

The node positions of petioles with bud(s) were recorded with node 1 arbitrarily assigned to the petiole base (or expanded leaf) closest to the shoot apex (Snow & Snow, 1932). On intact rhizomes, croziers were ignored, and on both intact and decapitated rhizomes, node 1 was assigned to the youngest fully expanded leaf on the plant. For each stipular bud, the location with respect to the proximal (near the rhizome) and distal (toward the leaf-tip) stipule margins (Fig. 1, 2) was also noted. Buds were scored as arrested (very small, with no sign of expansion) or expanded (larger in size, possibly with roots or leaves).

Data analysis.—Non-parametric Kruskal-Wallis ANOVA and Mann-Whitney U-tests were used to compare differences in bud counts among treatments and start dates. All statistical tests were run on an IBM PC using the STATISTIX 1.1 package (NH Analytical Software, 1986).

RESULTS

Bud location and frequency.—Leaves (whole and broken) were present at the first three to eight nodes examined on experimental plants of *Danaea wendlandii* (numbering the nodes starting with the youngest expanded leaf, as described above). Beyond that point, toward the basal end of the rhizome, leaves had abscised, leaving only petiole bases with attached stipules (Fig. 1). At the bases of petioles, arrested or expanded buds occurred only on the stipules, near one or more of the four points of attachment of the stipular flap margins to the petiole (Fig. 2). Arrested buds were so small and located so deep in the fold of stipular tissue against the petiole base at distal attachment points, or so close to the petiole of the nearest younger leaf base at proximal attachment points, that they could be seen only after dissection of the leaf base. Expanded buds (Fig. 2b) were clearly visible with a dissecting microscope.

Bud expansion.—Removal of shoot apices in *D. wendlandii* resulted in the appearance of expanded buds on stipules (Table 1). There were significant differences among treatments in the number of expanded buds per plant (Table 1; Kruskal-Wallis non-parametric ANOVA, $P = 0.0002$). Within treatments, differences in the number of buds for the staggered treatment start dates were not significant (Kruskal-Wallis non-parametric ANOVA for each treatment,

$P > 0.25$). Decapitation without additional treatment increased the number of visible buds 50-fold compared to intact plants (Table 1; Mann-Whitney U-test, $P = 0.0000$). Although a larger number of expanded buds were seen at bases of petioles of plants in which plain lanolin paste had been applied to cut apices, the difference between decapitation with and without plain lanolin application was not significant (Table 1; Mann-Whitney U-test, $P = 0.6239$).

Replacement of shoot apices with 1% IAA in lanolin resulted in a trend toward reduction in bud expansion compared to replacement with plain lanolin, but the difference between the two treatments was not significant (Table 1; Mann-Whitney U-test, $P = 0.6239$). Decapitated plants treated with IAA produced significantly more expanded buds than intact control plants (Table 1; Mann-Whitney U-test, $P = 0.0000$). Only one of the 18 intact plants had a single expanded bud (Table 1).

For all treatments, both arrested and expanded buds were more likely to occur near the proximal stipule margins than at the distal locations (Fig. 3). The position on the rhizome of nodes at which buds appeared on petiole bases varied with the treatment (Fig. 4), as did the proportion of those buds which were arrested (Fig. 4a) or expanded (Fig. 4b) at each node.

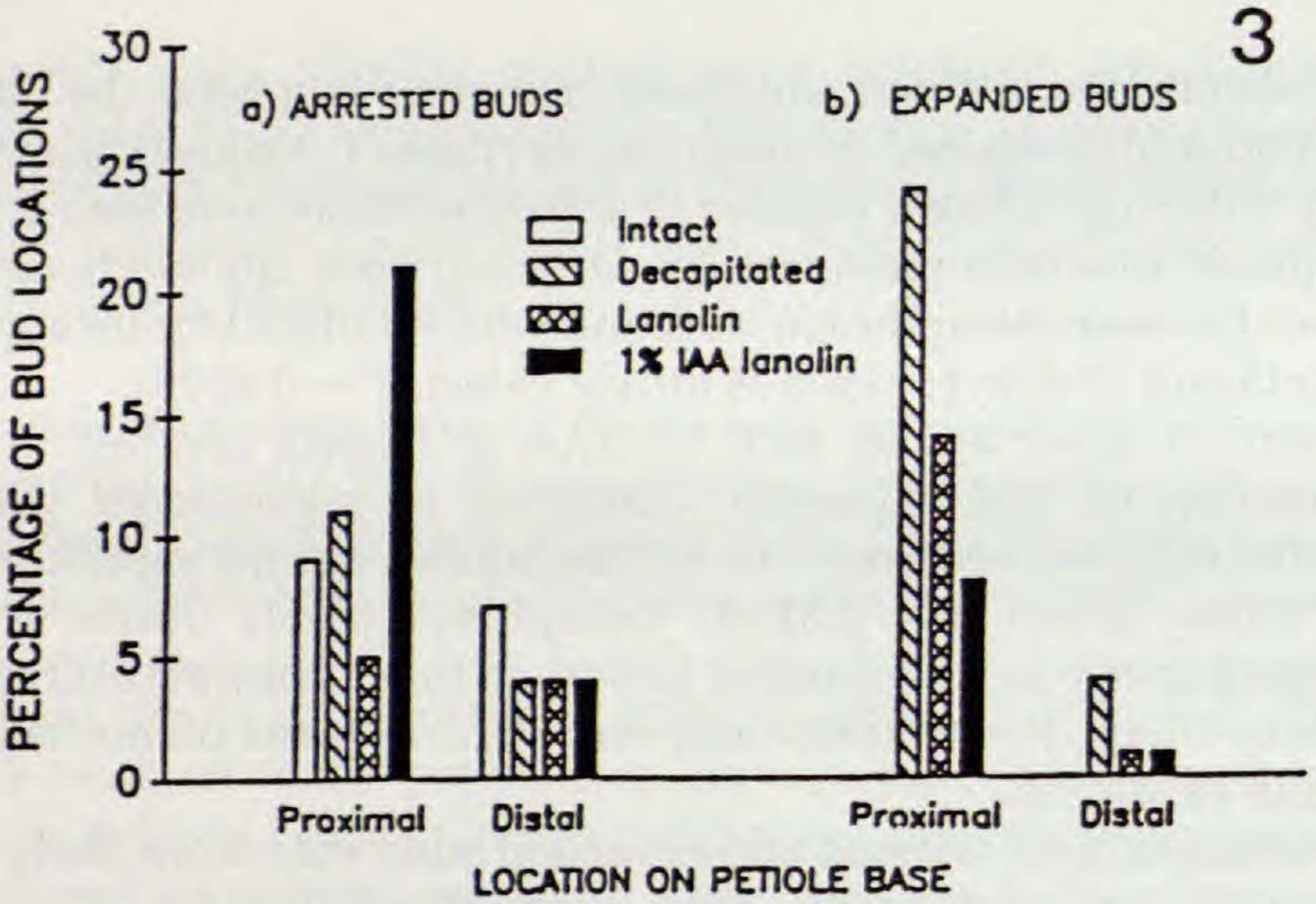
DISCUSSION

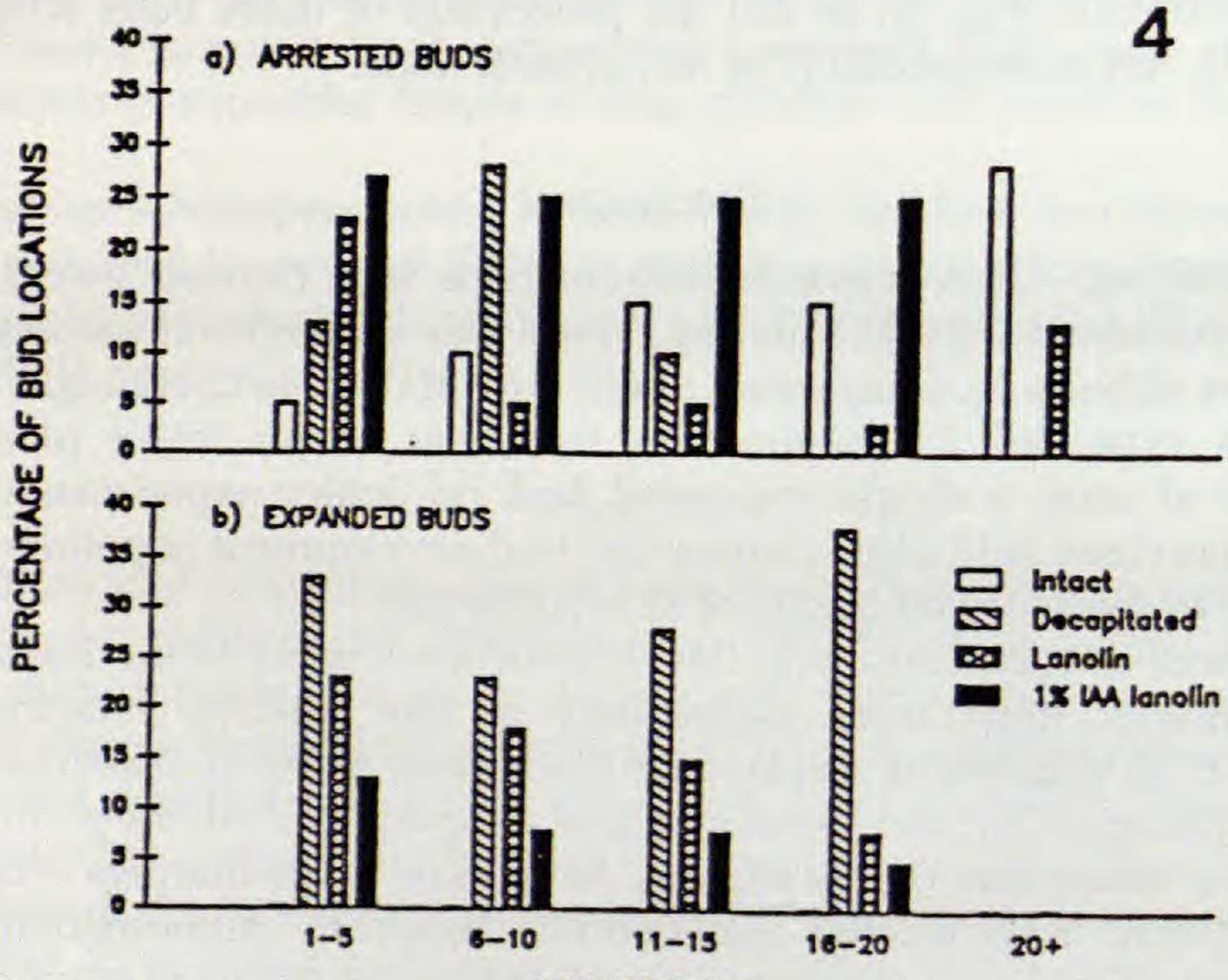
Stipular budding.—This research demonstrates that *Danaea wendlandii* is capable of extensive stipular budding. There was a significant increase in the total number of buds on decapitated plants with sixteen to thirty-eight times as many buds expanded in response to treatment as on intact plants. The appearance of only a single expanded bud on intact experimental plants confirmed previous field observations that bud development at petiole bases is uncommon in undisturbed plants of *D. wendlandii.*

Sites for bud development near the rhizome are limited to the leaf stipules. On the stipules, buds were found only at four specific locations. Bud development on stipules of *Angiopteris evecta* was noted by Bierhorst (1971). West (1917) located the meristematic cells of incipient buds in the petiole tissue of *D. nodosa,* rather than on the stipules. In contrast to the findings of Gwynne-Vaughan (1905), who located meristematic tissue in *Archangiopteris* and *Kaulfussia* (= *Christensenia*) at only the two proximal points of stipule margin attachment, our results show that buds of *D. wendlandii* can occur at the two distal points of stipule attachment as well.

Inhibition of budding.—Locations of leaf bases with stipular buds showed a distinct pattern along the length of the rhizome. Arrested buds were present on intact plants, with a greater number observed at the basal end of the rhizome than closer to the shoot apex, suggesting a pattern of apical dominance. The bud location pattern seen in intact plants was reversed for untreated and lanolin-treated decapitated plants, with twice as many buds near the apex as at the basal end of the rhizome, perhaps indicating a release from apical dominance.

Early studies of *Botrychium, Helminthostachys, Osmunda, Ophioglossum*





FIGS. 3–4. Comparison of petiole base locations and node positions of arrested and expanded stipular buds on rhizomes of *Danaea wendlandii*. Intact plants (N = 29 nodes) had no treatment. Decapitated plants (N = 34 nodes) had the shoot apex removed but no further treatment. Lanolin (N = 41 nodes) and 1% IAA in lanolin (N = 48 nodes) replaced decapitated apices in those treatments. Percentage of the total number of potential bud locations (4 per node) is indicated. Treatment percentages total less than 100% because not all potential sites had buds. Note that no intact plants had expanded buds. FIG. 3. Comparison of arrested (a) and expanded (b) stipular bud locations with respect to the location on the petiole bases of *D. wendlandii*. Proximal locations are at stipule margins nearest the rhizome, while distal locations are toward the apex of the leaf and on the abaxial side of the petiole base. FIG. 4. Comparison of arrested (a) and expanded (b) stipular buds with respect to node position on rhizomes of *D. wendlandii*. Node position 1 is the youngest expanded leaf, closest to the shoot apex.

and *Pteris,* reviewed by White (1979), document the variety of budding responses seen in ferns. Wardlaw (1943a, b, 1946) determined that apical dominance was responsible for inhibition of bud development in *Matteuccia struthiopteris, Dryopteris aristata,* and *Onoclea sensibilis.* This is in contrast to the findings of Hirsch (1975), who showed that decapitation of shoot apices of *Microgramma vaccinifolia* resulted in no release of arrested lateral buds. Although auxin has been suggested to have a role in inhibiting lateral bud development (White, 1979), our experiments with *Danaea wendlandii* are inconclusive, due to the limited auxin application regime. Other factors in exogenous hormone application experiments reviewed by Hillman (1984) may also have promoted the expansion of buds. These could include effects of wounding by decapitation and lack of water loss following lanolin application.

Associated leaf and root tissue may also contribute to inhibition of budding at petiole bases, as demonstrated for *Marsilea* (Laetsch & Briggs, 1963). The observed pattern of greater bud development at the basal end of intact rhizomes may result from added inhibition of budding by the upper petiole, rachis, laminar tissue and perhaps even developing leaf-tip buds of whole, unabscised leaves present closer to the apex. Leaf-associated inhibition could also account for the limited bud development seen at distal stipule margins compared to those proximal to the rhizome.

On decapitated plants of *D. wendlandii,* about 40% of the potential bud locations had buds (arrested or expanded), compared to 16% on intact plants. It appears that expansion of buds does not necessarily occur at all nodes. For example, at nodes 16–20 on decapitated plants, 38% of potential budding sites have buds. Since all of these buds have completely expanded, and no intermediate stages of budding are observed, it appears that only specific nodes have the capacity for bud initiation. This limitation may be related to the horizontal orientation of the rhizome, which would cause about half the potential budding locations to be underground, a condition which may inhibit bud initiation. In future experiments, the orientation of the rhizome should be noted when scoring bud development.

CONCLUSIONS

Expansion of stipular buds at petiole bases of *D. wendlandii* is rarely seen in the rain forest. Since bud formation and expansion can easily be induced experimentally, it appears that under natural conditions, some inhibitory mechanism is strong in this species or shoot apices of *D. wendlandii* rarely encounter conditions which trigger budding. Morphological and anatomical investigations comparing *D. wendlandii* with other *Danaea* species in which petiolar budding is more common in the field would be interesting. It may be that *D. wendlandii* has more effective structures for protecting the shoot apex (e.g. thick stipules, over-arching leaves). It is also possible that the leaf-tip budding phenomenon seen in *D. wendlandii* in some way inhibits budding along the rhizome. There may also be differences in distribution and development of underlying meristematic tissues. Comparative field studies may reveal subtle habitat differences among species which release or inhibit bud development (e.g., levels of litterfall, predation). Although the potential for substantial stipular budding is present in *D. wendlandii,* this phenomenon appears to have a limited role in the biology of this leaf-tip budding species in its rain forest habitat. Nonetheless it is potentially useful for horticultural or experimental propagation of this species, which like many other tropical ferns, is not easily obtained for study in temperate regions.

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